

such that the dye groups self-quench their fluorescence by dye stacking or dimerization, and

- D1
- b) contacting said substrate with a substance being assayed to determine the presence of an enzyme capable of cleaving an enzymatically cleavable bond wherein the enzymatic cleaving of said cleavable bond of the peptide will release the fluorescent dye groups from dye stacking or dimerizing, thereby producing an at least 20-fold increase in fluorescence intensity over that of the quenched dye groups thereby indicating the presence of said enzyme, wherein the emission wavelength of the fluorescent dye groups is at or above 570 nm.

D2

12. (Four times amended) A protease substrate comprising a flexible peptide and including two identical fluorescence dye groups that are drawn together by free energy attractions so as to self-quench fluorescence of the dye groups by intramolecular dimerization or stacking and which, when separated, fluoresce at an at least 20-fold increase in fluorescence intensity over that of the quenched dye groups, wherein the emission wavelength of the fluorescent dye groups is at or above 570 nm.

D3

21. (Four times amended) An assay method of detecting a microorganism, which microorganism produces a characteristic enzyme, comprising:

- a) providing an enzyme substrate specific for said characteristic enzyme produced by said microorganism comprising two or more identical fluorescence dye groups bound to a flexible peptide comprising one or more bonds cleavable by said characteristic enzyme, the dye groups being drawn together by free energy